BILD2

cobas®

Bilirubin Direct Gen.2 (Doumas standardization)

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05168384 190*	Bilirubin Direct Gen.2 (500 tests)	System-ID 03 7479 0	Roche/Hitachi **cobas c** 701/702
05168384 214*	Bilirubin Direct Gen.2 (500 tests)	System-ID 03 7479 0	Roche/Hitachi **cobas c** 701/702

Materials required (but not provided):

10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
10158046 122	Precibil (4 x 2 mL)	Code 306	

* Some kits shown may not be available in all countries.

English

System information

DBIL2: ACN 8735

Intended use

In vitro test for the quantitative determination of direct bilirubin in serum and plasma on Roche/Hitachi **cobas c** systems.

Summary[1]

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.

Test principle

Diazo method.[2]

Conjugated bilirubin and δ-bilirubin (direct bilirubin) react directly with 3,5 Dichlorophenyl diazonium salt in acid buffer to form the red-colored azobilirubin.

bilirubin + 3,5 DPD ⟶ azobilirubin

The color intensity of the red azo dye formed is directly proportional to the direct (conjugated) bilirubin concentration and can be determined photometrically.

Remark: Under the influence of blue light, e.g. during phototherapy of newborn children, unconjugated bilirubin is partly transformed into a water-soluble isomer called photobilirubin, a substrate for direct bilirubin tests. This fraction is detected by BILD2 and may lead to above-normal results in healthy children.

Reagents - working solutions

R1 Phosphoric acid: 85 mmol/L; HEDTA: 4.0 mmol/L; NaCl 50 mmol/L; detergent; pH 1.9

R2 3,5 Dichlorophenyl diazonium: 1.5 mmol/L; pH 1.3

R1 is in position B and R2 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

BILD2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label
On-board in use and refrigerated on the analyzer:	6 weeks
On-board on the Reagent Manager:	24 hours

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum: Collect serum using standard sample tubes.
Plasma: Li-heparin, K_2-, K_3-EDTA plasma.

Protect specimens from exposure to light.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability:[a),3,4]	2 days at 20-25 °C
	7 days at 4-8 °C
	6 months at -20 °C

a) If care is taken to prevent exposure to light

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

BILD2

Bilirubin Direct Gen.2 (Doumas standardization)

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 701/702 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 7-9	
Wavelength (sub/main)	800/546 nm	
Reaction direction	Increase	
Units	µmol/L (mg/dL, mg/L)	
Reagent pipetting		Diluent (H_2O)
R1	120 µL	–
R2	24 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	6.7 µL	–	–
Decreased	3.4 µL	–	–
Increased	6.7 µL	–	–

Calibration

Calibrator	S1: H_2O
	S2: Calibrator f.a.s.
Calibration mode	Linear regression
Calibration frequency	2-point calibration - after reagent lot change - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the manual test performance using the Doumas method.[5]

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	µmol/L x 0.0585 = mg/dL
	mg/dL x 10 = mg/L
	mg/dL x 17.1 = µmol/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at a direct bilirubin concentration of 34.2 µmol/L (2.0 mg/dL).

Hemolysis:[6] No significant interference up to an H index of 25 (approximate hemoglobin concentration: 15.5 µmol/L or 25 mg/dL).

Lipemia (Intralipid):[6] No significant interference up to an L index of 750. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[7,8]

Exception: Phenylbutazone causes artificially low bilirubin results.

Samples containing indocyanine green must not be measured.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result for the total bilirubin should be reported for both direct bilirubin and total bilirubin values.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on Roche/Hitachi **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

1.2-236 µmol/L (0.07-13.8 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.8 µmol/L (0.05 mg/dL)
Limit of Detection	= 1.2 µmol/L (0.07 mg/dL)
Limit of Quantitation	= 1.2 µmol/L (0.07 mg/dL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a precision of 20 % CV. It has been determined using low concentration direct bilirubin samples.

Expected values

Direct bilirubin ≤ 3.4 µmol/L (≤ 0.20 mg/dL)[1]

An upper limit of 10 µmol/L (0.59 mg/dL) direct bilirubin for neonates has been cited in the literature, although this has not been confirmed by internal data.[10]

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements with repeatability (n = 21) and intermediate precision

(2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	12.7 (0.74)	0.1 (0.01)	0.9
Precipath U	34.2 (2.0)	0.1 (0.01)	0.3
Human serum 1	4.0 (0.23)	0.1 (0.00)	1.9
Human serum 2	152 (8.9)	0.3 (0.02)	0.2
Human serum 3	231 (13.5)	0.6 (0.04)	0.3

Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L (mg/dL)	*µmol/L (mg/dL)*	*%*
Precinorm U	12.0 (0.70)	0.3 (0.02)	2.6
Precipath U	31.4 (1.8)	0.4 (0.02)	1.4
Human serum 1	1.5 (0.09)	0.2 (0.01)	10.0
Human serum 2	145 (8.5)	2.1 (0.12)	1.5
Human serum 3	211 (12.3)	3.2 (0.19)	1.5

Results for intermediate precision were obtained on the master system **cobas c** 501 analyzer.

Method comparison

Bilirubin values for human serum and plasma samples using with the Roche BILD2 reagent (ACN 735) on a Roche/Hitachi **cobas c** 501 analyzer (x) were compared to those determined with the same reagent on a Roche/Hitachi **cobas c** 701 analyzer (y).

Sample size (n) = 67

Passing/Bablok[11]	Linear regression
y = 1.018x + 0.45 µmol/L	y = 1.018x + 0.45 µmol/L
τ = 0.970	r = 1.000

The sample concentrations were between 3.0 and 208 µmol/L (0.18 and 12.2 mg/dL).

References

1 Balistreri WF, Shaw LM. Liver function. In: Tietz NW, ed. Fundamentals of Clinical Chemistry. 3rd ed. Philadelphia: WB Saunders 1987;729-761.

2 Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colorimeter. J Biol Chem 1937;119:481-490.

3 Quality of Diagnostic Samples, Recommendations of the Working Group on Preanalytical Quality of the German Society for Clinical Chemistry and Laboratory Medicine, 3rd completely revised ed. 2010.

4 WHO Publication: Use of anticoagulants in diagnostic laboratory investigations, WHO/DIL/LAB/99.1 Rev.2:Jan 2002.

5 Doumas BT, Perry BW, Jendrzejczak B, et al. Pitfalls in the American Monitor Kit Methods for Determination of Total and Direct Bilirubin. Clin Chem 1982;28(11):2305-2308.

6 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

7 Breuer J. Report on the Symposium “Drug effects in Clinical Chemistry Methods”. Eur J Clin Chem Clin Biochem 1996;34:385-386.

8 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

9 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

10 Soldin JS, Brugnara C, Wong EC. Pediatric Reference Intervals. AACC Press, 5th ed., 2005.

11 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606